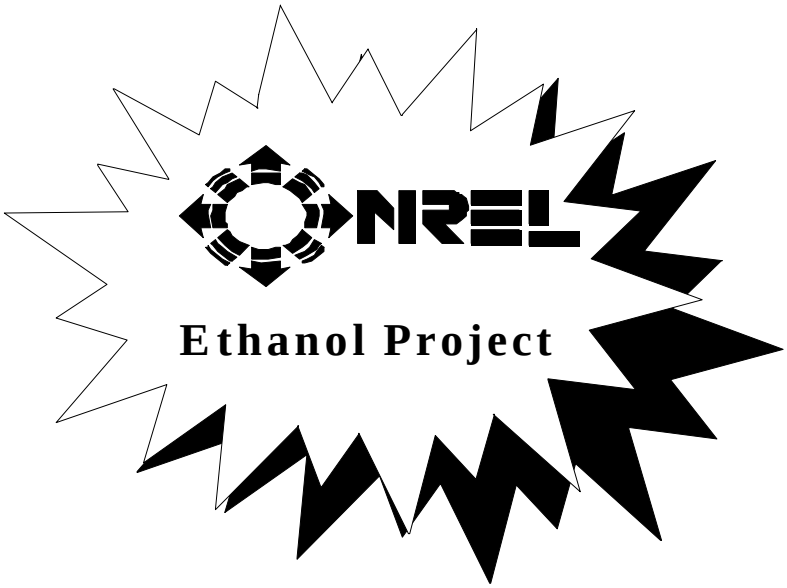

Chemical Analysis and Testing Task

Laboratory Analytical Procedure

LAP-017

Procedure Title: Determination of O-Acyl Groups in Biomass by High Performance Liquid Chromatography

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Determination of O-Acyl Groups in Biomass by High Performance Liquid Chromatography

Laboratory Analytical Procedure #017

1. Introduction

1.1 Aliphatic groups in wood and herbaceous feedstocks are acetyl and formyl groups which can be combined as O-acyl groups with the polysaccharide portion. A number of different approaches can be used to analyze for acetyl, including acid hydrolysis, saponification, transesterification, spectrophotometirc, and aminolysis. Acid hydrolysis was selected as the approach of choice since the hydrolyzate produced during the routine compositional analysis of cellulosic samples could also be used in the analysis of O-acyl groups. In this approach, dilute acid is used to split O-acyl groups from the polysaccharides. The resulting acetic and formic acids are then quantified by HPLC.

2. Scope

2.1 This procedure describes a HPLC method for determining the amount of acetyl and formyl groups cleaved upon hydrolysis of a biomass sample. The protocol utilizes the hydrolyzate generated by LAP-002, "Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography". Special handling during the post-autoclave steps of LAP-002 is required to ensure the volatile components are not lost.

2.2 Sample material suitable for this procedure include hard and soft woods, herbaceous materials (such as switchgrass and sericea), agricultural residues (such as corn stover, wheat straw, and bagasse), and waste-paper (such as office waste, boxboard, and newsprint). Pretreated biomass may also be analyzed by this method, although most pretreatment conditions will have already removed the acetyl and formyl groups. All results are reported relative to the 105EC oven-dried weight of the sample. In the case of extracted materials, the results may also be reported on an extractives-free basis.

2.3 All analyses shall be performed according to the guidelines established in the Ethanol Project Quality Assurance Plan (QAP).

3. References

3.1 Solar, R., F. Kacik, and I. Melcer. 1987. Simple Semimicro Method for the Determination of O-Acetyl Groups in Wood and Related Materials. Nordic Pulp and Paper Research Journal, 4:139-141.

3.2 Ethanol Project Laboratory Analytical Procedure #001, "Standard Method for the Determination of Total Solids in Biomass".

3.3 Ethanol Project Laboratory Analytical Procedure #002, "Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography".

3.4 Ethanol Project Laboratory Analytical Procedure #003, "Determination of Acid-Insoluble Lignin in Biomass".

3.5 Ethanol Project Laboratory Analytical Procedure #004, "Determination of Acid-Soluble Lignin in Biomass".

3.6 NREL Ethanol Project Laboratory Analytical Procedure #010, "Standard Method for the Determination of Extractives in Biomass".

4. Terminology

4.1 Prepared Biomass - Biomass that has been prepared by lyophilization, oven drying, air drying, and in some instances by extraction, to reduce the moisture content of the sample so it is suitable for O-acetyl group analysis.

4.2 Oven-Dried Weight - The moisture-free weight of a biomass sample as determined by LAP-001, "Standard Method for Determination of Total Solids in Biomass".

5. Significance and Use

5.1 The percent acetyl and formyl group contents are used in conjunction with other assays to determine the total composition of biomass samples.

6. Interferences

6.1 Formic acid is produced not only from the cleaving of O-formyl groups but also from the hydrolysis of HMF. The amount of levulinic acid present may be used as a rough indicator of the source of the formic acid, since levulinic acid is produced in equal molar quantities with formic acid when HMF is hydrolyzed.

7. Apparatus

7.1 Hewlett Packard Model 1090 HPLC, or equivalent, with refractive index detector.

7.2 HPLC column, BioRad Aminex@ HPX-87H (or equivalent).

7.3 Guard column, cartridges appropriate for the HPLC column used.

7.4 Analytical balance readable to 0.1 mg.

8. Reagents and Materials

8.1 Reagents

8.1.1 High purity chemicals for standards (98%+) - two sets of acetic acid, formic acid, and levulinic acid from different lots or manufacturers.

8.1.2 0.01 N H_2SO_4.

8.1.3 Water, 18 megohm deionized.

8.2 Materials

8.2.1 Disposable nylon syringe filters, 0.2 μm.

8.2.2 Disposable syringes, 3 mL.

8.2.3 Autosampler vials, with crimp top seals to fit.

9. ES&H Considerations and Hazards

9.1 Follow all applicable NREL Laboratory Specific Hygiene Plan guidelines.

9.2 Use caution when handling hot glass bottles after the autoclave step, as they may have become pressurized.

10. Sampling, Test Specimens and Test Units

10.1 The hydrolyzates generated as part of LAP-002 or LAP-003 are to be utilized in this method. Sample weight, total solid content, and extractive content (if needed) are determined as specified in the LAP-002 or LAP-003 protocols. The unused portion of the hydrolyzate can be taken through the LAP-002 and LAP-003 as desired.

11. Procedure

11.1 Upon removal of the hydrolyzed biomass samples from the autoclave (as part of the LAP-002 or LAP-003 procedure), allow the contents of the bottles to cool for 20 minutes at room temperature. Swirl the contents of each bottle vigorously and allow to cool further, until the contents are at room temperature and the solids have settled.

11.2 Working with one bottle at a time, remove the seal and stopper, taking care not to stir up any solids which have settled to the bottom.

11.3 Using a disposable syringe, immediately remove about 1.5 mL of sample, again taking care not to disturb the settled solids. Quickly pass this aliquot through a 0.2 μm filter into an autosampler vial. Immediately cap the vial.

11.4 Prepare a series of calibration standards in deionized water at concentrations appropriate for creating a calibration curve for each component of interest. A suggested scheme for the HPX-87H column is to prepare a set of multi-component standards containing acetic acid, formic acid, and levulinic acid in the range of 0.02 to 0.5 mg/mL.

11.5 Prepare an independent calibration verification standard (CVS) using chemicals obtained from a source other than that used in preparing the calibration standards. The CVS must contain precisely known amounts of each component contained in the calibration standards, at a concentration that falls in the middle of the validated range of the calibration curve. The CVS is to be analyzed after each calibration curve and at regular intervals in the HPLC sequence, bracketing groups of samples. The CVS is used to verify the quality of the calibration curve(s) throughout the HPLC run.

11.6 Analyze the calibration standards, the CVS, the MVS, and the samples by HPLC using a Biorad Aminex@ HPX-87H column. The following instrumental conditions are used:

Sample volume: 50 FL.
Eluant: 0.2 μm filtered and degassed 0.01 N H_2SO_4.
Flow rate: 0.6 mL/min.
Column temperature: 55°C.
Detector: refractive index.
Run time: 20 minutes (refer to precautionary note which follows).

Note: The hydrolyzates being tested will contain low levels of HMF and/or furfural. These components will appear as peaks in the chromatogram of the following sample. It is important to verify the HMF and furfural peaks are not interfering with the peaks of interest. With the instrument set up used to develop this method, use of a 20 minute run time resulted in the HMF peak appearing at about 10 minutes into the following chromatogram, and the furfural peak appearing at about 19 minutes. Neither peak interfered with the analytes of interest.

12. Calculations

12.1 Create a calibration curve by linear regression analysis for each component to be quantified. From these curves, determine the concentration in mg/mL of the organic acids present in each solution analyzed by HPLC.

12.2 For lyophilized, air dried, or oven dried samples, or for samples requiring no preparation, calculate the percentage of each organic acid present in the sample on an as received 105°C dry weight basis follows:

$$\%\ Analyte = \frac{C\ x\ \frac{1\ g}{1000\ mg}\ x\ V_F}{W_1\ x\ \frac{\%T_{as\ received}}{\%T_{prep}}}\ x\ 100\% = \frac{C\ x\ \frac{1\ g}{1000\ mg}\ x\ V_F}{W_1\ x\ \frac{\%T_{final}}{100\%}}\ x\ 100\%$$

Where: W_1 = initial weight of sample, in grams
V_F = volume of filtrate, 87.0 mL
C = concentration of component in hydrolyzed sample in mg/mL
$\%T_{as\ received}$ = % total solids content of the original sample (prior to any preparation steps) on a 105°C dry weight basis, as determined by the LAP-001
$\%T_{prep}$ = % total solids content of the sample as determined during the preparation of the sample for analysis (by lyophilization, oven-drying, or air drying)
$\%T_{final}$ = % total solids content of the prepared sample used in this analysis, on a 105°C dry weight basis, as determined by the LAP-001

Note: If the sample used in the analysis required no preparation (analyzed as received), then $\%T_{prep} = 100\%$ and $\%T_{final} = \%T_{as\ received}$.

12.3 If the biomass was prepared according to the "Standard Method for the Determination of Ethanol Extractives in Biomass" (LAP-010), first calculate the percentage of each organic acid on an extractives-free 105°C dry weight basis and then correct this value to an as received (whole sample) 105°C dry weight basis.

12.3.1 Calculate the percentage of each component on an extractives-free basis as follows:

$$\%\ Analyte_{extractives\text{-}free} = \frac{C \times \frac{1\ g}{1000\ mg} \times V_F}{W_1 \times \frac{\%T_{final}}{100\%}} \times 100\%$$

Where: C = concentration of component in hydrolyzed sample in mg/mL
V_F = volume of filtrate, 87.0 mL
W_1 = initial weight of extracted sample, in grams
$\%T_{final}$ = % total solids content of the prepared sample used in this analysis (in this case, extracted sample), on a 105°C dry weight basis, as determined by LAP-001

12.3.2 Correct the % organic acid value on an extractives-free basis, calculated above, to an as received (whole sample) 105EC dry weight basis as follows:

$$\%\ Analyte_{whole\ sample} = \%\ Analyte_{extractives\text{-}free} \times \frac{(100\% - \%\ extractives)}{100\%}$$

Where: % $Analyte_{extractives\text{-}free}$ = % analyte on an extractives-free 105°C dry weight basis, as determined in the previous step
% extractives = % extractives in the extracted sample as described in the Standard Method for the Determination of Extractives in Biomass (LAP-010)

13. Report

13.1 Report the percent of each analyte to two decimal places, on a whole sample 105°C dry weight basis or on an extractives-free basis. Cite the basis used in the report.

13.2 For replicate analyses of the same sample, report the average, standard deviation, and relative percent difference (RPD).

14. Precision and Bias

14.1 Data obtained by replicate testing of a hybrid poplar in two laboratories, using an HPX-87H column, gave a standard deviation in acetic-acid content of 0.08% and a CV% of 1.77%.

15. Quality Control

15.1 *Reported significant figures:* Report the percentage of each analyte present in the hydrolyzed sample to two decimal places, on a whole sample 105°C dry weight basis or on an extractives-free basis. Cite the basis used in the calculation.

15.2 *Replicates:* At minimum, all samples and the method verification standard are to be analyzed in duplicate.

15.3 *Blank:* The only requirement is a reagent blank specified in LAP-002 and LAP-003, which starts out as an empty 16x100 mm test tube (ie, no sample) which is taken through all the procedural steps.

15.4 *Relative percent difference criteria:* The RPD for acetic-acid must be less than 2.75%. If the RPD is too large, the sample must be rerun.

15.5 *Method verification standard:* A method verification standard must be run in duplicate with every batch. This method utilizes a well characterized standard material suitable for analysis. For example, NIST 8492 (*Populus deltoides*) is used as the MVS in O-acyl analysis of hardwoods.

15.6 *Calibration verification standard:* Calibration verification standards shall be independently prepared and analyzed as described in section 11.5 of this procedure.

15.7 *Sample size:* As specified in LAP-002 and LAP-003, a minimum of 0.6 grams of sample (on a dry weight basis) are required for duplicate analyses. If there is insufficient sample, the result will be flagged and the lack of precision will be noted.

15.8 *Sample storage:* Samples shall be stored in an airtight container and refrigerated.

15.9 *Standard storage:* Standards should be kept frozen in airtight vials or test tubes. Vortex the standards vigorously upon thawing to ensure thorough mixing.

15.10 *Standard preparation:* Standards are prepared as described in section 11.4 of this method.

15.11 *Definition of a batch:* Any number of samples which are analyzed and recorded together. The maximum size of a batch would be limited by the equipment constraints. A batch cannot be larger than what is practical with the equipment.

15.12 *Control charts:* The result of each replicate analysis of the method verification standard is recorded along with the average, RPD, and a laboratory book/page reference. The average value obtained for each analysis of the method verification standards is to be control charted.